

CLAIMS

1. A variant of an alpha-amylase having at least 60% homology to SEQ ID NO.8, comprising an alteration at one or more positions
5 selected from the group of:

49, 60, 104, 132, 161, 170, 176, 179, 180, 181, 183, 200, 203,
204, 207, 212, 237, 239, 250, 280, 298, 318, 374, 385, 393, 402,
406, 427, 430, 440, 444, 447, 482,

wherein

10 (a) the alteration(s) are independently

(i) an insertion of an amino acid downstream of the amino acid which occupies the position,

(ii) a deletion of the amino acid which occupies the position, or

15 (iii) a substitution of the amino acid which occupies the position with a different amino acid,

(b) the variant has alpha-amylase activity, and

(c) each position corresponds to a position of the amino acid sequence of the alpha-amylase having the amino acid sequence

20 shown in SEQ ID NO: 8.

2. The variant of claim 1, which variant has one or more of the following mutations: T49I; D60N; N104D; E132A,V,P; D161N; K170Q; K176R; G179N; K180T; A181N; D183N; D200N; X203Y; D204S; 25 D207V,E,L,G; X212I; K237P; S239W; E250G,F; N280S; X298Q; L318M; Q374R; E385V; Q393R; Y402F; H406L,W; L427I D430N; V440A; N444R,K; E447Q,K; Q482K using SEQ ID NO: 8 for the numbering.

3. The variant of claim 1 or 2, wherein the variant has the following mutations: K170Q+D207V+N280S; E132A+D207V; D207E+E250G+H406L+L427I; D207V+L318M; D60N+D207V+L318M; T49I+E132V+V440A; T49I+K176R+D207V+Y402F; Q374R+E385V+Q393R;

5 N190F+A209V+Q264S; G48A+T49I+G107A+I201F; T49I+G107A+I201F; G48A+T49I+I201F; G48A+T49I+G107A; T49I+I201F; T49I+G107A; G48A+T49I;

N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444K+E447Q+Q482K;

10 D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444K+E447Q+Q482K;

D161N+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444K+E447Q+Q482K;

D161N+A181N+D183N+D200N+D204S+K237P+S239W+H406W+

15 D430N+E447Q+Q482K;

N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+E447Q+Q482K;

D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+E447Q+Q482K;

20 N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N;

D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N;

H406W+D430N; N444K+E447Q+Q482K; E447Q+Q482K;

25 N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444R+N444K+E447K+Q482K;

D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W+H406W+D430N+N444R+N444K+E447K+Q482K;

N104D+D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W;

D161N+G179N+K180T+A181N+D183N+D200N+D204S+K237P+S239W;
H406W+D430N; N444K+E447K+Q482K; E447K+Q482K;
N104D+D161N+A181N+D183N+D200N+D204S+K237P+S239W;
N104D+D161N+A181N+D183N+D200N+D204S+K237P;
5 N104D+D161N+A181N+D183N+D200N+D204S;
D161N+A181N+D183N+D200N+D204S+K237P+S239W;
D161N+A181N+D183N+D200N+D204S+K237P;
D161N+A181N+D183N+D200N+D204S; K237P+S239W, using SEQ ID NO: 8
for the numbering.

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4. The variant of any of claims 1-3, wherein the parent alpha-amylase is derived from a strain of *B. licheniformis* (SEQ ID NO: 8), *B. amyloliquefaciens* (SEQ ID NO: 10), or *B. stearothermophilus* (SEQ ID NO: 6).

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5. The variant of any of claims 1-4, wherein the parent alpha-amylase is any of:

LE174; LE174+G48A+T49I+G107A+I201F; LE174+M197L;
LE174+G48A+T49I+G107A+M197L+I201F.

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6. The variant of claim 1, wherein the variant is mutated in one or more of the following positions: T51I; D62N; N106D; D134A,V,P; D163N; X172Q; K179R; G184N; K185T; A186N; D188N; D205N; M208Y; D209S; X212V,E,L,G; L217I, K242P, S244W, N255G,F,
25 N285S, S303Q, X323M; D387V, N395R; Y404F; H408L,W; X429I; D432N; V442A; X446R,K; X449Q,K; X484K, using SEQ ID NO: 4 for the numbering.

7. The variant of claim 1 or 6, wherein the variant has the following mutations: E212V+N285S; D134A+E212V; N255G+H408L+X429I; E212V+X323M; D62N+E212V+X323M; T51I+D134V+V442A; T51I+K179R+E212V+Y404F; D387V+N395R; 5 N195F+X212V+K269S, when using SEQ ID NO: 4 for the numbering.

8. The variant of any of claims 1-7, wherein the parent alpha-amylase is selected from the group comprising: SEQ ID NO: 2; SEQ ID NO: 4; SEQ ID NO: 12; SEQ ID NO: 13; or KSM-AP1378.

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9. The variant of any of claims 1-8, wherein the parent alpha-amylase is any of: SEQ ID NO. 4+D183*+G184*; SEQ ID NO. 4+D183*+G184*+N195F; SP722+D183*+G184*+M202L; SEQ ID NO. 4+D183*+G184*+N195F+M202L; SEQ ID NO. 6+I181*+G182*; SEQ ID 15 NO. 6+I181*+G182*+N193F; SEQ ID NO. 6+I181*+G182*+M200L; SEQ ID NO. 6+I181*+G182*+N193F+M200L; SEQ ID NO. 12+D183*+G184*; SEQ ID NO. 12+D183*+G184*+N195F; SEQ ID NO. 12+D183*+G184*+M202L; SEQ ID NO. 12+D183*+G184*+N195F+M202L.

20 10. The variant of any of claims 1-9, wherein the parent alpha-amylase has an amino acid sequence which has a degree of identity to SEQ ID NO: 8 of at least 70%, more preferably at least 80%, even more preferably at least about 90%, even more preferably at least 95%, even more preferably at least 97%, and even more 25 preferably at least 99%.

11. The variant of any of claims 1-10, wherein the parent alpha-amylase is encoded by a nucleic acid sequence, which hybridizes

under low, preferably medium, preferred high stringency conditions, with the nucleic acid sequence of SEQ ID NO: 7.

12. The variant of any of claims 1-11, which variant has altered
5 stability, in particular at high temperatures from 70-120°C and/or low pH in the range from pH 4-6

13. A DNA construct comprising a DNA sequence encoding an alpha-amylase variant according to any one of claims 1-12.

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14. A recombinant expression vector which carries a DNA construct according to claim 13.

15. A cell which is transformed with a DNA construct according
15 to claim 13 or a vector according to claim 14.

16. The cell according to claim 15, which is a microorganism, preferably a bacterium or a fungus.

20 17. The cell according to claim 16, which cell is a gram-positive bacterium, such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus laetus* or *Bacillus thuringiensis*.

25 18. A composition comprising an alpha-amylase variant of any of claims 1-12.

19. The composition of claim 18, further comprising a *B. stearothermophilus* alpha-amylase, particular in a ratio of 1:10 to 10:1, preferably 1:2.

5 20. The composition of claim 18 or 19, wherein the composition further comprises a glucoamylase, pullulanase and/or a phytase.

21. A detergent composition comprising an alpha-amylase variant according to any of claims 1-12.

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22. A detergent composition of claim 21, which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme, glucoamylase, maltogenic amylase, CGTase, mannanase, cutinase, laccase and/or a cellulase.

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23. Use of an alpha-amylase variant according to any of claims 1-12 or a composition according to any of claims 18-20 for starch liquefaction.

20 24. Use of an alpha-amylase variant according to any of claims 1-12 or a composition according to claims 18-20 for ethanol production.

25 25. Use of an alpha-amylase variant according to any one of claims 1-12 or a composition according to claims 18-20 for washing and/or dishwashing.

26. Use of an alpha-amylase variant of any one of claims 1-12 or a composition according to claims 18-20 for textile desizing.